Synthesis and Biological Activities of New Conformationally Restricted Analogues of (−)-Indolactam-V: Elucidation of the Biologically Active Conformation of the Tumor-Promoting Teleocidins

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Abstract: The tumor-promoting teleocidins and their core structure (−)-indolactam-V (**1**) exist in two stable conformers in solution at room temperature. The *cis* amide assumes a twist conformation while the *trans* amide exists in a sofa form. In order to identify the biologically active conformation of the teleocidins, we have synthesized new twist-restricted analogues **5a** and **6** based on an aza-Claisen rearrangement of (−)-N^{13}-desmethyl-N^{13}-allylindolactam-V (**3**) and a sofa-restricted analogue, (−)-5-methylindolactam-V (**22**). The activities of these new compounds were evaluated in three *in vitro* bioassays associated with *in vivo* tumor-promoting activity: binding to the protein kinase C regulatory domain, induction of the Epstein−Barr virus early antigen, and stimulation of radioactive inorganic phosphate incorporation into phospholipids of HeLa cells. These three biological activities correlated well for each derivative. Twist-restricted analogues **5a** and **6** showed significant activities in the three assays, comparable to **1** itself. In contrast, sofa-restricted **22** showed little activity related to tumor promotion. Introduction of a prenyl group into position 7 or 18 of **5a** and **6** significantly enhanced the activity while sofa-restricted (−)-5-prenylindolactam-V (**23**) showed only very weak activity. These results indicate that the active conformation of the teleocidins and **1** is close to the twist form. This is the first evidence bearing on the active conformation of the teleocidins based on conformationally restricted analogues *with an intact indolactam skeleton* and is in accord with conclusions reported for benzolactams, analogues without the pyrrole moiety. This study also describes the synthesis of new biologically active compounds (**26a**, **26b**, **28**) based on inactive (+)-epiindolactam-V (**24**), involving a further application of the aza-Claisen rearrangement. Bridge formation between positions 5 and 13 of indolactam derivatives represents a particularly effective analogue design strategy, allowing for the remote control of the conformation of this ring system and for the introduction of a wide range of structural variations, as required for the development of new protein kinase C activators with high isozyme selectivity.

Introduction

(−)-Indolactam-V (**1**),[1,2] the core structure of tumor-promoting teleocidins,[3] is a key compound for investigating the structural requirements for the activation of protein kinase C (PKC), a crucial enzyme involved in cellular signal transduction and in a variety of normal and abnormal cellular processes (Figure 1).[4] Over 100 derivatives of this core structure have been synthesized and assayed during the last decade, allowing for the identification of several structural features that are required for the potent activity exhibited by this class of compounds.[5] Conformational studies have also been undertaken, leading to the finding that the teleocidins and **1** exist in two stable conformers in solution at room temperature, with the *cis* amide in a twist conformation and the *trans* amide in a sofa form (Figure 2).[6] Of particular importance to the further development of these biological probes and medicinal leads is the identification of the conformation required for activity. Such studies are also of great value in efforts to elucidate the common

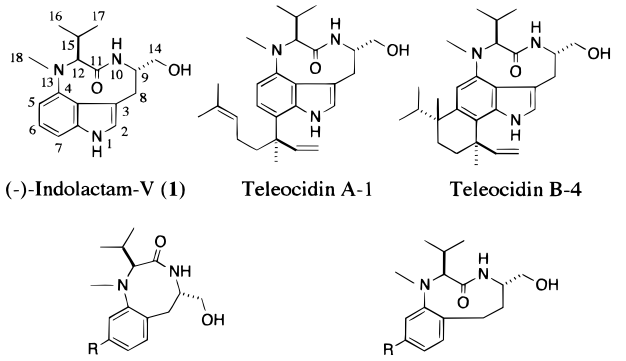

Figure 1. Structures of teleocidin-related compounds and benzolactams.

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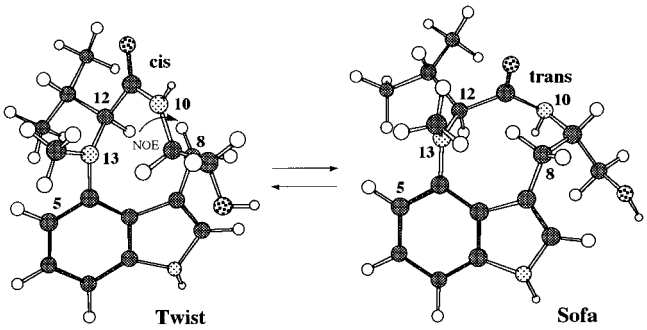

Figure 2. Conformation of (−)-indolactam-V (**1**).[6]

structural basis for the significant tumor-promoting activities exhibited by these compounds and other structurally different promoters, such as the highly potent phorbol esters, information that would facilitate the identification of tumor promoters, augment the development of cancer prevention protocols, and enhance understanding of the mechanism(s) of tumor promotion at the molecular level.[7−14]

The design and synthesis of conformationally restricted analogues of **1** represent one of the most promising approaches to the identification of the biologically active conformation. Pertinent to such studies is the general observation that 8-membered lactams and their smaller ring counterparts exist in a *cis* amide conformation while lactams of 10 or more members prefer the *trans* amide conformation.[15,16] For nine-membered lactams, the *cis* and *trans* amide isomers are typically similar in energy, as exemplified by the teleocidins which exist as an equilibrium mixture of the two conformers. Drawing on these observations, Endo *et al.*[17,18] have recently synthesized teleocidin analogues in which the indole subunit is replaced by an aromatic ring. The 8-membered benzolactam-V8 was designed as a *cis* amide-restricted analogue while the 9- and 10-membered benzolactam-V9 and -V10 were selected as *trans* amide-restricted analogues (Figure 1). Although these compounds were almost inactive, introduction of an *n*-decyl group on the benzene ring of benzolactam-V8 resulted in the appearance of significant activity in HL-60 cells, suggesting that the active conformation of the teleocidins is close to the twist form of the *cis* amide. Kozikowski *et al.*[19] have also synthesized a nine-membered benzolactam similar to benzolactam-V9 and showed that the sofa conformation of *trans* amide is not the biologically active form.

Our own studies in this area were directed at the design and synthesis of conformationally restricted analogues that incorporate *an intact indolactam skeleton*. This approach serves to maintain the structural features of teleocidin required for molecular recognition while providing for remote control over their spatial orientation (conformation) as required for systematic comparisons with the lead indolactam-containing compounds in this series. Retention of the intact indolactam recognition surface of the teleocidins in these analogues also provides the potential for preserving potency which is often attenuated in ring-modified systems such as benzolactam-V8-310, a less active analogue of **1** in PKC activation.[18] This approach has led to the design and synthesis of new twist-restricted analogues **5a** and **6** by aza-Claisen rearrangement of (−)-N^{13}-desmethyl-N^{13}-allylindolactam-V (**3**).[20] A full report on the synthesis and biological activities of these (**5a**, **6**) and other new twist-restricted analogues (**11**, **12**, **14**) along with sofa-restricted ones, (−)-5-methylindolactam-V (**22**) and (−)-5-prenylindolactam-V (**23**), is described herein. The effectiveness of remote control of recognition element conformation in this series is further illustrated in the case of the newly synthesized analogues **26a**, **26b**, and **28** which while incorporating the complete structure of the *inactive* (+)-epiindolactam-V (**24**) are themselves biologically active. Overall, these studies show that the active conformation of the teleocidins and **1** is the twist form of the *cis* amide and provide a flexible strategy for the design of new analogues.

Results and Discussion

Design and Synthesis of Conformationally Restricted Analogues of (−)-Indolactam-V (1). The conformational equilibrium of **1** is mainly attributed to a *cis*−*trans* isomerization of the nine-membered lactam. For the purposes of analogue design, it is noteworthy that, in the sofa conformation of the *trans* amide, the methyl group at position 13 is almost perpendicular to the indole ring, while in the twist conformation of the *cis* amide, the methyl group and the indole ring are closer to a planar arrangement (Figure 2).[6] These observations suggest that bridge formation between positions 5 and 13 of **1** could be used to control the conformation of the indolactam, providing a potentially effective and tunable approach to twist-restricted analogues of **1**. Substitution on this bridge could also be used to fine-tune conformational features and to regulate transport and potentially isozyme selective recognition, as required for the development of novel biological probes and medicinal leads.

Recently, Beholz and Stille[21] reported a noteworthy systematic investigation of the aza-Claisen rearrangement of *N*-alkyl-*N*-allylanilines,[22] showing that the course and efficiency of this reaction are influenced by Lewis acid catalyst type, stoichiometry, and concentration. Under suitable conditions, the initially formed rearrangement products were found to cyclize to indole and indoline derivatives. The corresponding aza-Claisen rearrangement of (−)-N^{13}-desmethyl-N^{13}-allylindolactam-V (**3**; cau-

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Table 1. Effect of Lewis Acids on the Aza-Claisen Rearrangement of (−)-N^{13}-Allyl-N^{13}-desmethylindolactam-V (**3**) and its 14-*O*-Acetate[a]

	product formation (yield %)	
reagent (equiv)	**4**	14-*O*-acetate of **4**
$AlCl_3$ (0.35)	23	nt[b]
$AlCl_3$ (0.45)	36	34
$AlCl_3$ (0.7)	0	0
$ZnCl_2$ (0.7)	26	0
$ZnCl_2$ (0.9)	22	nt
2N H_2SO_4 (1.2)	0	nt

[a] Rearrangements were run at 0.5 M of **3** or its 14-*O*-acetate in xylene (except 2 N H_2SO_4) at 140 °C for 20 min in a sealed tube. [b] Not tested.

tion[23]) provides the basis for our approach to the synthesis of twist-restricted analogues of **1**.

Compound **3** was derived from allylation of (−)-N^{13}-desmethylindolactam-V (**2**), which in turn was synthesized from L-tryptophan by the method of Nakatsuka *et al.*,[24] Kogan *et al.*,[25] and Endo *et al.*[6] with slight modifications. Allylation was achieved by treatment of **2** with allyl bromide in methanol containing $NaHCO_3$, which gave the *N*-allyl product **3** in 35% yield.[26] The direct transformation of analogous *N*-alkyl-*N*-allylanilines to indole and indoline derivatives is reported to be favored by a high concentration of substrate (0.5−1.0 M) and less than a stoichiometric amount of a Lewis acid.[21] Consequently, attempts were made initially to transform *N*-allylindolactam **3** directly into the conformationally restricted analogues **5** and **6** by heating (140 °C) a 0.5 M solution of **3** in xylene for 20 min in the presence of various Lewis acids. Of the Lewis acids surveyed, $AlCl_3$ and $ZnCl_2$ were the most effective reagents for promoting [3,3] rearrangement of **3** (Table 1). The use of 0.7 equiv of $AlCl_3$ completely decomposed the products. Reactions conducted with protic acid (e.g., H_2SO_4) were complicated presumably by cleavage of the lactam.

Although the reactions conducted with 0.7 or 0.9 equiv of $ZnCl_2$ gave only the rearrangement product **4**, the desired rearrangement and *ortho* cyclization products **5a**, **5b**, and **6** were obtained albeit in low yield when 0.35 or 0.45 equiv of $AlCl_3$ was used (Figure 3). The acetate of **5b** was correlated with **6** by DDQ oxidation and hydrolysis (70%). The 14-chlorinated compound **7** and the cyclization product **8**, which is deduced to arise from **7** by dehydrochlorination followed by intramolecular addition, were also isolated as byproducts. Compound **8** has an unique skeleton similar to D-lysergic acid diethylamide (LSD), a strong hallucinogen. The absolute stereochemistry at position 9 was not determined. The complementary C-9 epimer was also detected in trace amounts (<0.2%). Since the free hydroxyl group at C-14 of **3** was significantly chlorinated under the reaction conditions, the 14-*O*-acetate of **3** was also subjected to the aza-Claisen rearrangement. However, the yield of the 14-*O*-acetate of **4** did not increase significantly even when 0.45 equiv of $AlCl_3$ was used, as shown in Table 1. Furthermore, *ortho* rearrangement products like **5a**, **5b**, and **6** were not isolated in this case.

Since the yield for the one-step conversion of **3** to the conformationally restricted analogue **6** was too low (0.6%) to be used for bioassays and subsequent prenylation, our efforts focused on the cyclization of **4**, the major product formed in the rearrangement of **3**. For this purpose, the palladium-mediated intramolecular amination procedure reported by the Hegedus group[27] proved to be highly effective. Cyclization of 14-*O*-acetate of **4** in the presence of a catalytic amount (0.1 equiv) of $PdCl_2(CH_3CN)_2$, 1 equiv of benzoquinone, and 10 equiv of LiCl in THF gave **9** in 67% yield. A stoichiometric amount of $PdCl_2(CH_3CN)_2$ in THF, followed by triethylamine addition, also provided **9** but in lower yield (32%).

The extension of this cyclization procedure to the synthesis of six-membered-ring analogues like **20** and **21** was also investigated. Hegedus *et al.*[27] reported that 2-(2′-butenyl)aniline cyclized to the six-membered-ring product under catalytic conditions without LiCl, reflecting a preference for amination at the more substituted terminus of the olefin. Therefore, (−)-5-(2′-butenyl)-N^{13}-desmethylindolactam-V (**16**) was acetylated and the resulting acetate subjected to the catalytic cyclization using 0.1 equiv of $PdCl_2(CH_3CN)_2$ and 1 equiv of benzoquinone in the absence of LiCl. However, only the five-membered-ring product **18** was isolated (24%). A similar reaction involving the pentenyl derivative **17** produced only a trace of cyclized product. The failure of these reactions to produce six-membered-ring analogues could be attributed to the instability of compounds like **20** and **21** arising from strain or steric interactions caused by the adjacent indole ring, the nine-membered lactam, or the isopropyl group at position 12.

The ^{1}H NMR spectrum of **5a** in deuteriochloroform showed that it existed only as a single conformer in solution. This conformer was assigned the twist structure by comparison with several characteristic signals and coupling constants exhibited by the two conformers of **1** (Table 2). The NOE difference spectrum of **5a** was also in accord with this assignment. Saturation of H-12 (δ 4.33) resulted in a strong enhancement (13%) of the H-8b signal (δ 3.21) as observed for the twist conformer of **1**.[6] The absolute configuration at position 19 was established as *R* by the NOE difference spectra. Saturation of H-15 (δ 2.65) caused a significant enhancement (11%) of the H-19 signal (δ 4.04); no NOE enhancement except H-18a (δ 2.58) and H-19 (δ 4.04) was observed by saturation of H_3-20 (δ 1.19). Compound **6** also existed only in a twist conformation (Table 2). A significant NOE enhancement (average 13%) between H-8b (δ 3.50) and H-12 (δ 5.35) of **6** is consistent with this assignment. The use of deuteriochloroform for the NOE studies is to model the hydrophobic binding site of PKC−phosphatidylserine aggregate. Although protic solvent such as methanol decreased significantly the ratio of the twist conformer of **1**,[6] **5a** and **6** existed only in a twist conformation even in methanol.

The ^{1}H NMR signals of **5b**, the C-19 epimer of **5a**, were very broad at room temperature but became sharp when measured at −10 °C. Compound **5b** exists as two conformers (∼1:1), which due to severe steric interaction between the isopropyl group at position 12 and the methyl group at position 19 are neither of the sofa nor the twist form. The elucidation of these conformations remains to be investigated.

We have previously reported that 5-substituted indolactam derivatives such as (−)-5-acetylindolactam-V or (−)-5-fluoroindolactam-V exist exclusively in the sofa conformation and that they are essentially inactive.[28,29] However, the activities of these derivatives are expected to reflect both the electronic and hydrophilic effects of the substituents at position 5 in addition to the conformational change caused by steric effects.

(23) Caution: Indolactams are tumor promoters and should be handled with care.

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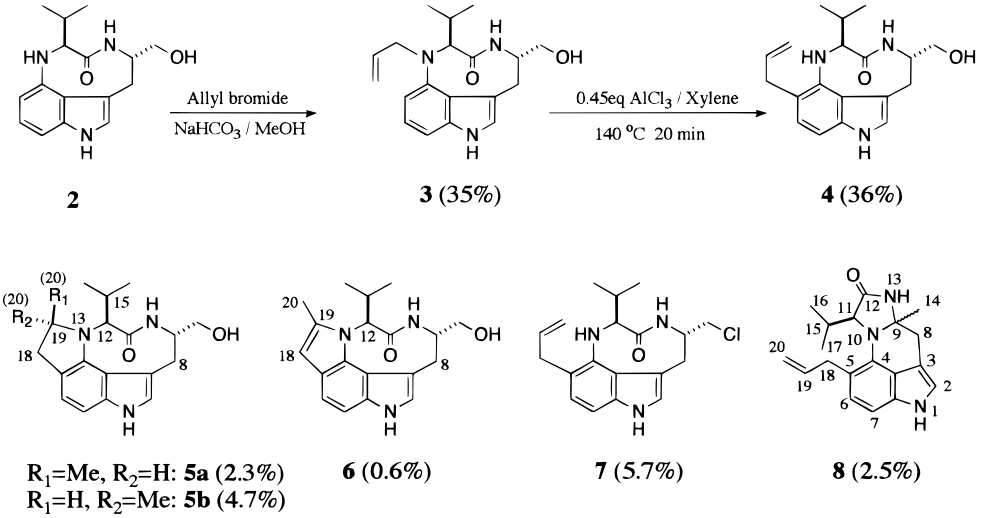

Figure 3. Aza-Claisen rearrangement of (−)-N^{13}-desmethyl-N^{13}-allylindolactam-V (**3**).

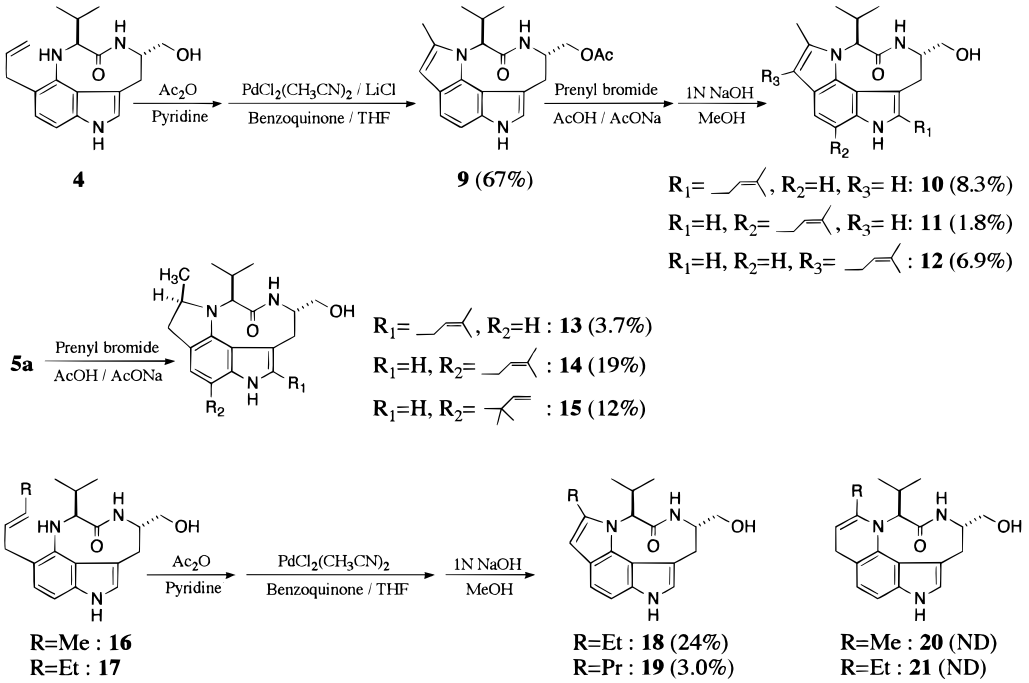

Figure 4. Synthesis of twist-restricted analogues with a prenyl group via palladium-assisted intramolecular amination.

Table 2. Several Characteristic Signals of the ^{1}H NMR Spectra of (−)-Indolactam-V (**1**), the *Ortho* Cyclization Products (**5a**, **6**), and (−)-5-Methylindolactam-V (**22**) in Deuteriochloroform (500 MHz)

	δ (multiplicity, J in Hz)				
	1				
position	twist conformer[a]	sofa conformer[a]	**5a** twist conformer[b]	**6** twist conformer[c]	**22** sofa conformer[d]
8a	3.01 (dd, J = 17.4, 3.8)	2.84 (dd, J = 14.4, 1.6)	2.92 (dd, J = 17.1, 4.3)	3.05 (dd, J = 17.0, 4.1)	2.78 (dd, J = 14.3, 1.5)
8b	3.20 (br d, J = 17.4)	3.11 (dd, J = 14.4, 4.9)	3.21 (br d, J = 17.1)	3.50 (br d, J = 17.0)	3.21 (dd, J = 14.3, 4.3)
10	6.59 (br s)	4.72 (d, J = 10.8)	6.48 (br s)	6.48 (br s)	4.91 (d, J = 10.7)
12	4.39 (d, J = 10.2)	2.99 (d, J = 10.8)	4.33 (d, J = 10.1)	5.35 (d, J = 11.0)	2.99 (d, J = 10.7)
14a	3.54 (m)	3.44 (m)	3.55 (m)	3.55 (m)	3.37 (m)
14b	3.74 (m)	3.44 (m)	3.71 (m)	3.72 (m)	3.37 (m)

[a] Sofa:twist = 1.0:2.6 (0.004 M, 27 °C). [b] Twist only (0.01 M, 27 °C). [c] Twist only (0.003 M, 27 °C). [d] Sofa >98% (0.02 M, 27 °C).

To further evaluate the activity of the sofa conformer, (−)-5-methylindolactam-V (**22**) and (−)-5-prenylindolactam-V (**23**) were selected for study as the sofa-restricted analogues (Figure 5). **22** was synthesized by reduction of (−)-14-*O*-acetyl-5-formylindolactam-V[30] with $LiAlH_4$ and $AlCl_3$ in THF, followed by alkaline hydrolysis (56% yield). **23** was obtained from **1** by the method reported previously.[31] Both compounds existed exclusively as single conformers in solution. Several characteristic signals and coupling constants (especially high-field shifts of H-10 and H-12) of **22** and **23** indicate that these compounds exist in the sofa conformation (Table 2 and Experimental Section). The most stable conformer of **22** is

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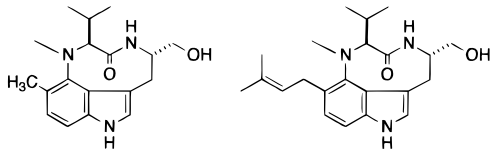
(31) Irie, K.; Kajiyama, S.; Funaki, A.; Koshimizu, K.; Hayashi, H.; Arai, M. *Tetrahedron* **1990**, *46*, 2773−2788.



(-)-5-Methyl-indolactam-V (**22**) (-)-5-Prenyl-indolactam-V (**23**)

Figure 5. Structure of sofa-restricted (−)-5-methylindolactam-V (**22**) and (−)-5-prenylindolactam-V (**23**).

predicted by Endo *et al.*[32] to be the sofa form based on a high-temperature molecular dynamics (HTMD) method.

The terpenoid side chain of the teleocidins is found to enhance their tumor-promoting activity.[33,34] Consequently, a prenyl group was introduced into various positions of the indole ring of the twist-restricted analogues (**5a**, **6**). Treatment of **9** with prenyl bromide in acetic acid−water medium buffered with sodium acetate, followed by alkaline hydrolysis,[31,35] gave 2-, 7-, or 19-prenylated derivatives (**10**−**12**) in 8.3, 1.8, and 6.9% yields, respectively (Figure 4). Similar reaction using **5a** gave three compounds **13**−**15** in 3.7, 19, and 12% yields, respectively. These prenylated compounds gave ^{1}H NMR spectra similar to those of **5a** and **6**. NOE experiments confirmed that these derivatives (**10**−**15**) exist in a twist form.

It is noteworthy that **15** with a 1,1-dimethylallyl group at position 7 like teleocidin A-1 was obtained in a yield of 12%. Direct S_N2' attack on a prenyl derivative by an indole is typically not favored.[31,35] It has been widely accepted that the 1,1-dimethylallyl group of several indole alkaloids like pendolmycin,[36] echinulin, and brevianamides[37] are biosynthesized via primary attack at N-1 of the indole ring by prenyl diphosphate with subsequent aza-Claisen rearrangement.[31,37] The significant formation of **15** suggests, however, that direct S_N2' attack at position 7 could be involved or that the N-1 alkylation and rearrangement is especially facile, findings of significance with respect to the biosynthesis of pendolmycin or teleocidin A-1.

Biological Activities of the Conformationally Restricted Analogues of 1. The biological activities of the conformationally restricted analogues of **1** were examined by three *in vitro* bioassays related to *in vivo* tumor promotion: binding to the PKC regulatory domain, the Epstein−Barr virus early antigen (EBV-EA)-inducing ability, and ability to enhance incorporation of radioactive inorganic phosphate ($^{32}P_i$) into phospholipids of HeLa cells (Tables 3 and 4). These three biological activities were found to correlate well for each derivative.

The binding affinity to PKC regulatory domain was evaluated by inhibition of the specific binding of [^{3}H]phorbol 12,13-dibutyrate (PDBu) to the regulatory domain of rat brain PKCγ (γ-Cys2) and mouse skin PKCη (η-Cys2). We have recently synthesized these domains consisting of ∼50 amino acid residues by solid phase synthesis and showed that they serve as effective surrogates for the so-called conventional PKCs (PKCα, β1, β2, γ)[38] and novel PKCs (PKCδ, ϵ, η, θ),[38] respectively.[39,40] Dose−response curves were plotted for each compound, and the concentration at which 50% of [^{3}H]PDBu binding was inhibited (IC_{50}) was determined. A computer program (Statistical Analysis System) with a probit (probability unit) procedure[41] was used for calculation of IC_{50}. The binding constant (K_i) was calculated from the IC_{50} and the dissociation constant (K_d) of PDBu to each model peptides (K_d for γ-Cys2, 44.1 nM; for η-Cys2, 0.91 nM)[40] by the method of Sharkey and Blumberg.[42] The K_d for η-Cys2 (0.91 nM) coincides well with that of native PKCη (0.87 nM).[43] However, various K_d values of PDBu in the presence of calcium and phosphatidylserine have been reported for native PKCγ: $K_d = 16$,[44] 2.4,[45] and 0.33 nM.[43] The K_d for γ-Cys2 (44.1 nM) coincides with that reported by Evans *et al.*[44] (16 nM) and with that of GST-Cys2 (carboxyl-terminal fusion protein of GST with the second PKCγ cysteine-rich region, amino acids 92−173) reported by Bell *et al.*[45] (23 nM), excluding our fault on the experimental procedure. Since conventional PKCs such as PKCγ have the calcium- and phosphatidylserine-binding domain (C_2 domain) close to the phorbol ester-binding domain (C_1 domain), the PDBu binding affinity to native PKCγ might be affected significantly by the C_2 domain, depending on the slightly different assay conditions employing calcium and phosphatidylserine, resulting in the variation of the K_d values. At present, the *true* K_d value of PDBu to native PKCγ is unknown.

EBVs are under the strict control of the host human lymphoblastoid Raji cells. They are activated by tumor promoters to produce the early antigen (EA).[46,47] The EBV-EA inducing activity is expressed as the percentage of EA-positive cells and the effective concentration required to induce EA production. In our experimental conditions, the potent tumor promoter teleocidin B-4 exhibited maximum induction between 30 and 40% at 10^{-7} M.[48] The effective concentration, EC_{50}, is defined as the concentration that induces half of the percentage of EA-positive cells induced at the optimum concentration. As most compounds showed a typical sigmoidal dose−response curve, the computer program with a probit procedure[41] was also used for calculation of EC_{50}.

We have also measured the stimulation of $^{32}P_i$ incorporation into HeLa cell phospholipids[49] for our analogues because the enhancement of phospholipid metabolism plays an important role in tumor promotion.[50] Teleocidin B-4 and **1** caused a maximum increase of ∼5−7-fold over the control (DMSO) at

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Table 3. PKC Binding and EBV-EA Inducing Activity of the Conformationally Restricted Analogues of (−)-Indolactam-V (**1**)

compound	binding to PKC [K_i (nM)][a]			% of EA-positive cells at the indicated concns[b]					effective concn[c] [EC_{50}(nM)]
	γ-Cys2	η-Cys2	ratio[d]	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5} M	
twist/sofa									
1	1030	3.36	306.5		1.1 (0.3)[e]	15.4 (1.0)	33.7 (3.1)	29.8 (0.0)	98.4
teleocidin B-4	2.0	0.1	20.0	17.1 (3.0)	24.8 (0.8)	37.0 (1.9)			1.8
twist only									
5a	234	14.0	16.7			1.2 (1.1)	14.1 (2.8)	16.0 (2.1)	∼1000
6	14179	114	124.4		1.1 (0.4)	12.7 (4.7)	34.0 (0.2)	36.1 (2.5)	147.9
14	143	1.0	143		1.4 (0.5)	13.8 (0.6)	23.9 (4.2)	13.2 (2.3)	72.4
11	93.2	0.86	108.4	2.2 (1.2)	14.9 (1.8)	25.1 (2.1)	31.0 (1.1)		13.8
12	62.4	1.34	46.6	1.7 (1.1)	27.6 (2.3)	29.2 (0.8)	20.0 (0.0)		3.2
10	27123	821	33.0				2.6 (0.4)	18.9 (7.1)	∼5000
sofa only									
22	140076	359	390.2			0.3 (0.4)	1.4 (0.5)	9.4 (2.1)	>10000
23	7495	13.5	555.2			2.6 (1.3)	10.8 (1.5)	23.9 (2.5)	∼2000
neither twist nor sofa									
5b	13700	439	31.2			0.2 (0.3)	0.8 (0.4)	17.8 (0.7)	∼10000

[a] This assay was carried out by the procedure of Sharkey and Blumberg[42] with slight modification. The assay solution (250 μL) consisted of 50 mM Tris-HCl (pH 7.4), 3 mg/mL bovine γ-globulin, 30 nM [^{3}H]PDBu, 50 μg/mL 1,2-di(*cis*-9-octadecenoyl)-*sn*-glycero-3-phospho-L-serine, 10 nM γ-Cys2, and various concentrations of an inhibitor. For the η-Cys2 binding assay, 20 nM [^{3}H]PDBu and 5 nM η-Cys2 were used. These PKC model peptides were folded by 5 molar equiv of $ZnCl_2$.[40] [b] This assay was done by the method reported previously[47] with slight modifications.[48] Sodium *n*-butyrate (3 mM) was added to all samples to enhance the sensitivity of Raji cells. The viability of the cells exceeded 80% in all experiments. Final DMSO concentration was 0.5%. [c] The effective concentration is defined as the concentration that induces half of the percentage of EA-positive cells induced at the optimum concentration. As most compounds showed a typical sigmoidal dose−response curve, a computer program (Statistical Analysis System) with a probit (probability unit) procedure[41] was used for calculation of EC_{50}. [d] ratio = K_i for γ-Cys2/K_i for η-Cys2. [e] Standard deviation.

Table 4. Incorporation of $^{32}P_i$ into Phospholipids of HeLa Cells by the Conformationally Restricted Analogues of (−)-Indolactam-V (**1**)[a]

compound	relative cpm[a]/mg of protein at the indicated concns					effective concn[b] [EC_{50} (nM)]
	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5} M	
twist/sofa						
1			1.65 (0.17)[c]	5.35 (0.16)	7.20 (1.35)	489
teleocidin B-4	1.31 (0.05)	2.29 (0.13)	5.92 (0.42)			13.8
twist only						
5a			1.27 (0.13)	1.92 (0.27)	3.07 (0.25)	∼10000
6			1.61 (0.20)	4.58 (0.88)	7.22 (0.14)	648
14			2.71 (0.00)	5.84 (0.49)	5.16 (0.23)	130
11	0.96 (0.14)	1.30 (0.03)	4.47 (0.15)	6.30 (0.39)		61.2
12	0.93 (0.01)	1.24 (0.05)	4.45 (0.22)	6.23 (0.38)		62.4
10				1.09 (0.19)	1.88 (0.01)	inactive
sofa only						
22				1.18 (0.08)	1.46 (0.14)	inactive
23				1.65 (0.28)	1.97 (0.01)	inactive
neither twist nor sofa						
5b			1.33 (0.01)	1.20 (0.00)	1.93 (0.38)	inactive

[a] This assay was done by the method reported previously[49] with slight modifications.[51] Final DMSO concentration was 0.2%. Radioactivity of 0.2% DMSO treated cells was determined to be 1.00. [b] The effective concentration is defined as the concentration that enhance half of the $^{32}P_i$ incorporation enhanced at the optimum concentration. As most compounds showed a typical sigmoidal dose−response curve, a computer program (Statistical Analysis System) with a probit (probability unit) procedure[41] was used for calculation of EC_{50}. [c] Standard deviation.

the optimum concentration, as shown in Table 4. The effective concentration, EC_{50}, was similarly calculated using the probit procedure.[41]

Among the compounds without a prenyl moiety (**5a**, **5b**, **6**, **22**), the binding affinity of sofa-restricted **22** to the PKC model peptides was extremely low, while twist-restricted **5a** and **6** had significant binding affinity. A similar tendency was observed in the EBV-EA induction test. **1** and twist-restricted **6** showed potent EBV-EA induction at 10^{-6} M. Twist-restricted **5a** also induced significant EBV-EA at 10^{-6} M. In contrast, **22** scarcely showed any induction at 10^{-6} M. Greater differences were observed in the $^{32}P_i$ incorporation into phospholipids of HeLa cells. Both **1** and **6** showed potent stimulation at 10^{-6} M, and **5a** showed weak but significant stimulation at 10^{-5} M. However, **22** was almost inactive even at 10^{-5} M. Compound **5b**, whose conformation was deduced to be neither the sofa nor the twist form, showed very weak activities in the three assays. These results indicate that the active conformation of the teleocidins and **1** is close to the twist form. This is the first evidence bearing on the active conformation of the teleocidins based on conformationally restricted analogues *with an intact indolactam skeleton* and is in accord with conclusions reported by Endo *et al.*[17,18] and Kozikowski *et al.*[19] for benzolactams, analogues without the pyrrole moiety.

Introduction of a hydrophobic prenyl group into the indole ring of twist-restricted **5a** and **6** significantly enhanced biological activities. Compounds **11**, **12**, and **14** had potent PKC binding affinities, *comparable to PDBu* (K_i for γ-Cys2, 52.9 nM; for η-Cys2, 0.6 nM).[40] Compounds **11** and **12** proved to be *more potent EBV-EA inducers than **1*** and enhanced significantly the $^{32}P_i$ incorporation into phospholipids of HeLa cells at 10^{-7} M. Compound **14** also showed significant activities in the EBV-EA induction and the $^{32}P_i$ incorporation at 10^{-6} M. On the other hand, sofa-restricted **23** bound to the PKC model peptides with affinities 10−50-fold less than the twist-restricted analogues (**11**, **12**, **14**). Compound **23** was a weak EBV-EA inducer and almost inactive in the $^{32}P_i$ incorporation. The weak but notable activity of **23** can be explained by conformational conversion upon binding to the receptor since the energy difference between the most stable sofa conformation and the twist conformation was estimated to be ∼3 kcal/mol by Endo *et al.*[32] These data also support the above conclusion that the active conformation

of teleocidins is close to the twist form, not to the sofa form. Compound **12**, in which the prenyl group is attached to position 18, had strong biological activities in the three assays. This is the first example of an indolactam analogue with enhanced activity arising from hydrophobic substitution at a site other than position 6, 7, or 12 of the indolactam skeleton.[33,51,52] Twist-restricted **10** with a prenyl group at position 2 was almost inactive in the three bioassays. This is attributed to the steric effect at position 2 as reported previously.[33]

With the discovery of at least 10 PKC isozymes in recent years,[38] increasing importance is placed on isozyme-specific analysis of function in order to elucidate the role of PKC in cellular signal transduction. Isozyme-selective agonists represent powerful tools for such studies, but the rational development of such compounds has been hampered by limited information on the solution structure of the phorbol ester−PKC−phosphatidylserine aggregate.[53−56] The relative affinities of the conformationally restricted analogues of **1** for the two different PKC isoform model peptides (γ-Cys2, η-Cys2) are expressed as a ratio of each K_i value in Table 3. Although *isozyme-specific* compounds were not identified in this study, the selectivity changed for each derivative in the range of 10-fold. Teleocidin B-4, **5a**, and **12** showed more selective binding to γ-Cys2 than to η-Cys2 relative to **1**, **6**, **11**, and **14**. These results suggest that new agents exhibiting improved levels of isoform selectivity are obtainable through structural modifications of **1**.

The three biological activities in Tables 3 and 4 correlate well for each derivative in general, but there are some exceptions. It is noteworthy that **1** and the twist-restricted **6** showed quite similar activities in EBV-EA induction and $^{32}P_i$ incorporation, while the binding ability of **6** to the PKC model peptides was about 15−30-fold weaker than that of **1**. Moreover, **5a** showed only 10-fold weaker activities than **1** in EBV-EA induction and $^{32}P_i$ incorporation although it bound 5-fold more strongly to γ-Cys2 than **1**. Since PKC plays an important role in these two *in vitro* bioassays, these differences are potentially attributable to the PKC isozymes other than PKCγ and PKCη in intact cells employed in these assays.

Attempts To Synthesize New Indolactam Congeners by Aza-Claisen Rearrangement. The above results indicate that bridge formation between positions 5 and 13 of the indolactams is an effective method to fix the molecule in an active conformation. As a further test of this approach to analogue design, we have investigated whether the inactive (+)-epiindolactam-V (**24**),[57] a C-12 epimer of **1**, could be rendered active through incorporation of a suitable bridge (Figure 6).

24 exists as two stable conformers, *r-cis*-sofa and *r*-sofa form, in solution at room temperature[32,58] and is completely inactive in several bioassays related to tumor promotion (Figure 7).[57] However, models suggest that bridge formation between positions 5 and 13 of **24** might induce a conformational preference similar to biologically active analogues. In order to test this point, (+)-epi-N^{13}-desmethyl-N^{13}-allylindolactam-V (**25**) was

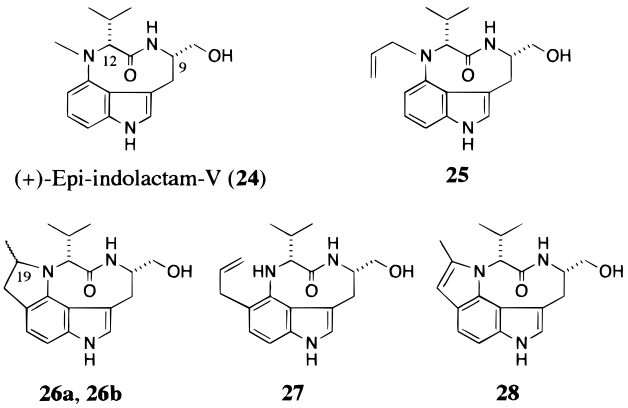

Figure 6. Structure of (+)-epiindolactam-V (**24**) and its *ortho* rearrangement products (**26a**, **26b**, **28**).

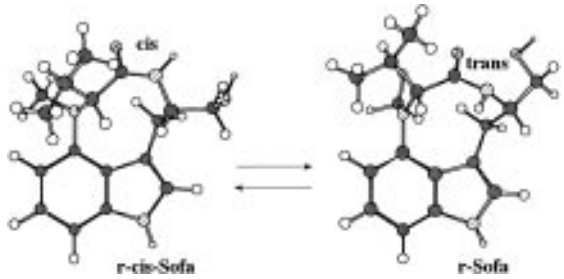

Figure 7. Conformation of (+)-epiindolactam-V (**24**).[32,58]

synthesized from (+)-epi-N^{13}-desmethylindolactam-V, which in turn was prepared from L-tryptophan by a previously reported method.[6,24] Treatment of **25** with 0.45 equiv of $AlCl_3$ under reaction conditions similar to those employed in the reaction of **3** gave three products, **26a**, **26b**, and **27** in 4.7, 4.1, and 22% yields, respectively. Compounds **26a** and **26b**, C-19 epimers, are designated respectively according to the order of the R_f value on silica gel TLC. Palladium-assisted intramolecular amination of **27** gave **28** in 39% yield.

The ^{1}H NMR spectrum of **28** in deuteriochloroform showed that **28** exists as a single conformer in solution. The fixed conformation was deduced to be close to the r-twist form on the basis of the low-field chemical shift of NH-10 (δ 6.40) and H-12 (δ 5.67), the coupling constant of NH-10 (0 Hz), and the NOE difference spectrum. A significant NOE enhancement between H-8b and H-12 supported the *r*-twist conformer.[58] **26b** existed mainly as one conformer, while **26a** existed as at least two conformers in solution. The specific conformations of **26a** and **26b** could not be determined by the NOESY spectra since each conformer interconverts too rapidly to be observable on the NMR time scale. The absolute configuration at C-19 of these compounds also remains to be elucidated.

The binding affinity to the PKC model peptides and EBV-EA inducing activity of these compounds (**26a**, **26b**, **28**) and **24** are summarized in Table 5. Although **24** was inactive in both assays, the two bridge modified analogues, C-19 epimers **26a** and **26b**, bound to the PKC model peptides with potencies similar to that of **6** (Table 3). Compound **28** also showed weak but significant binding to η-Cys2. In the EBV-EA induction test, **26b** and **28** proved to be significant inducers at 10^{-5} M, but **26a** was almost inactive. Compound **26a** might be a specific inhibitor in the EBV-EA induction by TPA-type tumor promoters since **26a** inhibited the specific [^{3}H]PDBu binding to the PKC model peptides. Although the conformation of **26a** and **26b** is not elucidated at present, it is evident that the bridge formation between positions 5 and 13 of **24** can be used to regulate biological activity. These results suggest that bridge formation between positions 5 and 13 of indolactam derivatives is an effective and tunable method for the design of new

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Table 5. PKC Binding and EBV-EA Inducing Activity of (+)-Epiindolactam-V (**24**) Derivatives

	binding to PKC [K_i (nM)][a]			% of EA-positive cells at the indicated concns[b]		
compound	γ-Cys2	η-Cys2	ratio[c]	10^{-7}	10^{-6}	10^{-5} M
24	>500000	>500000			1.5 (0.5)[d]	2.1 (0.1)
26a	8044	116	69.3	1.3 (0.0)	1.1 (0.7)	3.2 (1.2)
26b	5811	194	30.0	1.5 (0.0)	0.6 (0.4)	10.5 (0.5)
28	>500000	6383			4.2 (1.1)	19.2 (1.1)

[a] This assay was carried out by the procedure of Sharkey and Blumberg[42] with slight modification. The assay solution (250 μL) consisted of 50 mM Tris-HCl (pH 7.4), 3 mg/mL bovine γ-globulin, 30 nM [^{3}H]PDBu, 50 μg/mL 1,2-di(*cis*-9-octadecenoyl)-*sn*-glycero-3-phospho-L-serine, 10 nM γ-Cys2, and various concentrations of an inhibitor. For the η-Cys2 binding assay, 20 nM [^{3}H]PDBu and 5 nM η-Cys2 were used. These PKC model peptides were folded by 5 molar equiv of $ZnCl_2$.[40] [b] This assay was done by the method reported previously[47] with slight modifications.[48] Sodium *n*-butyrate (3 mM) was added to all samples to enhance the sensitivity of Raji cells. The viability of the cells exceeded 80% in all experiments. Final DMSO concentration was 0.5%. [c] ratio = K_i for γ-Cys2/K_i for η-Cys2. [d] Standard deviation.

medicinal leads and potentially isozyme selective agonists or antagonists of PKC.

Conclusion

We have synthesized new twist-restricted teleocidin analogues **5a**, **6**, **11**, **12**, and **14** via aza-Claisen rearrangement of (−)-N^{13}-desmethyl-N^{13}-allylindolactam-V (**3**) along with sofa-restricted ones (**22**, **23**) in order to identify the active conformation of **1** and the teleocidins. On the basis of the three *in vitro* bioassays (related to *in vivo* tumor-promoting activity) conducted on these analogues, it is concluded that the active conformation of **1** and the teleocidins is close to the twist form of *cis* amide, not to the sofa form of *trans* amide. This is the first evidence bearing on the active conformation of the teleocidins based on conformationally restricted analogues *with an intact indolactam skeleton* and is in accord with conclusions reported for benzolactams, analogues without the pyrrole moiety.[17−19] The present study significantly reduces the number of PKC-active conformational candidates[59] through remote conformational control of the indolactam ring. It also introduces an approach to analogue design that can be used to generate new biologically active compounds (**26a**, **26b**, **28**) even from inactive analogues such as **24**, thereby providing a particularly effective approach to new PKC activators potentially with isozyme selectivity. Further studies are focused on the synthesis of new active compounds from inactive benzolactam-V9[17,18] and (−)-indolactam-G[57] by the similar aza-Claisen rearrangement, allowing access to new highly rigid analogues for isoform-selective recognition.

Experimental Section

General Methods. The following spectroscopic and analytical instruments were used: UV, Shimadzu UV-200; $[\alpha]_D$, Jasco DIP-1000; ^{1}H and ^{13}C NMR, Bruker ARX500 (reference to TMS); HPLC, Waters Model 600E with Model 484 UV detector; (HR) EI-MS, JEOL JMS-DX300.

HPLC was carried out on a YMC packed A-023 (silica gel, 10 mm i.d. × 250 mm), AM-323 (ODS, 10 mm i.d. × 250 mm), AQ-323 (ODS, 10 mm i.d. × 250 mm) (Yamamura Chemical Laboratory), and μ-Bondasphere C_{18} (19 mm i.d. × 150 mm) column (Waters Associates). Wakogel C-100 and C-200 (silica gel, Wako Pure Chemical Industries) and YMC gel A60-350/250 (ODS, Yamamura Chemical Laboratory) were used for column chromatography.

[^{3}H]PDBu (20 Ci/mmol) was purchased from NEN Research Products. **1** and teleocidin B-4 were isolated from *Streptoverticillium blastmyceticum* NA34-17 as reported previously.[60] All other chemicals and reagents were purchased from chemical companies and were of special grade.

Synthesis of (−)-N^{13}-Desmethyl-N^{13}-allylindolactam-V (3). (−)-N^{13}-Desmethylindolactam-V (**2**; 534 mg, 1.86 mmol) was treated with allyl bromide (1.05 mL, 12.1 mmol) and $NaHCO_3$ (1.52 g, 18.1 mmol) in MeOH (10.3 mL) at room temperature for 19 h. After the reaction mixture was partitioned between EtOAc and water, the EtOAc layer was dried over Na_2SO_4, and concentrated *in vacuo* to dryness. The residue was purified by column chromatography on Wakogel C-100 using toluene and increasing amounts of acetone, followed by HPLC on μ-Bondasphere using 62.5% MeOH to give **3** (215 mg, 0.657 mmol) in 35% yield: $[\alpha]_D$ −32.1° (c = 0.28, MeOH, 21 °C); UV λ_{max} (EtOH) nm (ϵ) 293 (7000), 226 (25 000); ^{1}H NMR (500 MHz, $CDCl_3$, 0.045 M, 27 °C, sofa:twist = 1.0:1.1) twist conformer, δ 0.70 (d, J = 6.8 Hz, 3H, H_3-16 or 17), 0.93 (d, J = 6.3 Hz, 3H, H_3-16 or 17), 2.59 (m, 1H, H-15), 2.69 (br s, 1H, OH-14), 3.06 (dd, J = 17.4, 3.8 Hz, 1H, H-8a), 3.15 (br d, J = 17.4 Hz, 1H, H-8b), 3.56 (m, 1H, H-14a), 3.75 (m, 1H, H-14b), 3.88 (m, 1H, H-18a), 4.18 (dd, J = 15.8, 7.3 Hz, 1H, H-18b), 4.39 (d, J = 10.0 Hz, 1H, H-12), 4.45 (m, 1H, H-9), 5.04 (d, J = 10.2 Hz, 1H, H-20a), 5.22 (d, J = 17.1 Hz, 1H, H-20b), 5.72 (m, 1H, H-19), 6.54 (d, J = 7.8 Hz, 1H, H-5), 6.89 (s, 1H, H-2), 6.90 (d, J = 7.7 Hz, 1H, H-7), 7.03 (t, J = 7.9 Hz, 1H, H-6), 7.10 (br s, 1H, NH-10), 8.01 (br s, 1H, NH-1); sofa conformer, δ 0.94 (d, J = 6.5 Hz, 3H, H_3-16 or 17), 1.28 (d, J = 6.6 Hz, 3H, H_3-16 or 17), 1.48 (br s, 1H, OH-14), 2.38 (m, 1H, H-15), 2.84 (dd, J = 14.6, 1.4 Hz, 1H, H-8a), 3.02 (d, J = 10.9 Hz, 1H, H-12), 3.18 (dd, J = 14.6, 4.8 Hz, 1H, H-8b), 3.42 (m, 2H, H_2-14), 3.52 (dd, J = 15.2, 6.7 Hz, 1H, H-18a), 3.88 (m, 1H, H-18b), 4.45 (m, 1H, H-9), 4.77 (d, J = 10.0 Hz, 1H, H-20a), 4.83 (br d, J = 10.5 Hz, 1H, NH-10), 4.84 (d, J = 18.7 Hz, 1H, H-20b), 5.72 (m, 1H, H-19), 7.04 (s, 1H, H-2), 7.05 (dd, J = 7.0, 0.7 Hz, 1H, H-5), 7.15 (t, J = 7.7 Hz, 1H, H-6), 7.27 (d, J = 7.7 Hz, 1H, H-7), 8.35 (br s, 1H, NH-1); ^{13}C NMR (125 MHz, 0.37 M, 27 °C) twist conformer, δ 19.99 (C-16 or C-17), 21.72 (C-16 or C-17), 29.53 (C-15), 33.91 (C-8), 47.67 (C-18), 56.26 (C-9), 64.88 (C-14), 71.92 (C-12), 104.49 (C-7), 108.87 (C-5), 114.35 (C-3), 117.27 (C-20), 119.06 (C-3a), 121.60 (C-2), 122.17 (C-6), 134.92 (C-19), 139.34 (C-7a), 144.50 (C-4), 174.79 (C-11); sofa conformer, δ 19.74 (C-16 or C-17), 19.81 (C-16 or C-17), 25.35 (C-15), 28.69 (C-8), 51.59 (C-18), 54.90 (C-9), 62.35 (C-14), 77.74 (C-12), 108.26 (C-3), 110.47 (C-7), 114.99 (C-20), 122.39 (C-6), 124.11 (C-5), 125.57 (C-2), 127.71 (C-3a), 137.06 (C-19), 139.61 (C-7a), 143.21 (C-4), 173.42 (C-11), these assignments were derived from a ^{1}H−^{1}H COSY, C−H COSY, and COLOC spectrum; HR-EI-MS *m/z* 327.1943 (M^+, calcd for $C_{19}H_{25}N_3O_2$, 327.1948).

Aza-Claisen Rearrangement of 3. Compound **3** (147 mg, 450 μmol) was distributed in 10 vials. After the sample was dried over P_2O_5, $AlCl_3$ (2.7 mg, 20 μmol) and xylene (89.9 μL) were added to each vial containing **3** (14.7 mg, 45.0 μmol). Each vial was sealed tightly and heated at 140 °C for 20 min. The reaction was quenched by addition of saturated aqueous $NaHCO_3$ solution, and the mixture was extracted with EtOAc. After the EtOAc layer was dried over Na_2SO_4, the EtOAc extracts were purified by column chromatography on Wakogel C-100 using toluene and increasing amounts of acetone, followed by HPLC on μ-Bondasphere using 65% MeOH and on YMC A-023 using 75% *n*-hexane, 10% $CHCl_3$, and 15% 2-PrOH, to give **4** (53.0 mg, 162 μmol, 36%), **5a** (3.4 mg, 10 μmol, 2.3%), **5b** (6.9 mg, 21 μmol, 4.7%), **6** (0.9 mg, 3 μmol, 0.6%), **7** (8.8 mg, 26 μmol, 5.7%), and **8** (3.5 mg, 11 μmol, 2.5%).

Compound **4**: $[\alpha]_D$ −182.4° (c = 0.13, MeOH, 23 °C); UV λ_{max} (EtOH) nm (ϵ) 281 (6200), 229 (29 900); ^{1}H NMR (500 MHz, $CDCl_3$, 0.015 M, 27 °C, only one conformer) δ 1.06 (d, J = 6.8 Hz, 3H, H_3-16 or 17), 1.24 (d, J = 6.5 Hz, 3H, H_3-16 or 17), 2.23 (m, 1H, H-15), 2.54 (br s, 1H, OH-14), 2.88 (dd, J = 15.4, 9.4 Hz, 1H, H-8a), 3.08

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(dd, J = 15.4, 6.9 Hz, 1H, H-8b), 3.40−3.56 (m, 5H, H_2-18, H-12, H-14a, NH-13), 3.74 (m, 1H, H-14b), 4.97 (dd, J = 17.1, 1.7 Hz, 1H, H-20a), 5.07 (dd, J = 10.2, 1.7 Hz, 1H, H-20b), 5.42 (br s, 1H, H-9), 5.83 (d, J = 10.2 Hz, NH-10), 6.03 (m, 1H, H-19), 6.80 (s, 1H, H-2), 6.95 (d, J = 8.2 Hz, 1H, H-6 or 7), 7.03 (d, J = 8.2 Hz, 1H, H-6 or 7), 7.94 (br s, 1H, NH-1), these assignments were derived from a ^{1}H−^{1}H COSY spectrum; HR-EI-MS *m/z* 327.1946 (M^+, calcd for $C_{19}H_{25}N_3O_2$, 327.1948).

Compound **5a**: $[\alpha]_D$ −333.9° (c = 0.078, MeOH, 20 °C); UV λ_{max} (EtOH) nm (ϵ) 319 (8300), 288.5 (5900), 235 (22 900); ^{1}H NMR (500 MHz, $CDCl_3$, 0.01 M, 27 °C, twist only) δ 0.72 (d, J = 6.6 Hz, 3H, H_3-16 or 17), 1.00 (d, J = 6.3 Hz, 3H, H_3-16 or 17), 1.19 (d, J = 6.4 Hz, 3H, H_3-20), 2.05 (br s, 1H, OH-14), 2.58 (dd, J = 15.3, 3.7 Hz, 1H, H-18a), 2.65 (m, 1H, H-15), 2.92 (dd, J = 17.1, 4.3 Hz, 1H, H-8a), 3.21 (br d, J = 17.1 Hz, 1H, H-8b), 3.55 (m, 1H, H-14a), 3.63 (dd, J = 15.3, 9.8 Hz, 1H, H-18b), 3.71 (m, 1H, H-14b), 4.04 (m, 1H, H-19), 4.25 (br s, 1H, H-9), 4.33 (d, J = 10.1 Hz, 1H, H-12), 6.48 (br s, 1H, NH-10), 6.71 (d, J = 8.1 Hz, 1H, H-7), 6.85 (s, 1H, H-2), 6.91 (d, J = 8.1 Hz, 1H, H-6), 7.82 (br s, 1H, NH-1); ^{13}C NMR (125 MHz, 0.089 M, 27 °C, twist only) δ 18.42 (C-16 or 17), 21.36 (C-16 or C-17), 23.34 (C-20), 26.34 (C-15), 33.35 (C-8), 38.27 (C-18), 53.18 (C-19), 56.83 (C-9), 64.95 (C-14), 68.28 (C-12), 101.48 (C-7), 113.25 (C-3 and C-3a), 115.56 (C-5), 119.53 (C-6), 121.41 (C-2), 139.53 (C-7a), 147.25 (C-4), 175.70 (C-11), these assignments were derived from a ^{1}H−^{1}H COSY, NOESY, HMQC, and HMBC spectrum; HR-EI-MS *m/z* 327.1944 (M^+, calcd for $C_{19}H_{25}N_3O_2$, 327.1948).

Compound **5b**: $[\alpha]_D$ +27.4° (c = 0.11, MeOH, 20 °C); UV λ_{max} (EtOH) nm (ϵ) 317 (6500), 280 (4300), 235 (20 200); ^{1}H NMR (500 MHz, $CDCl_3$, 0.037 M, −10 °C, major:minor = 1.1:1.0) major conformer, δ 1.05 (d, J = 6.5 Hz, 3H, H_3-16 or H_3-17), 1.14 (d, J = 6.4 Hz, 3H, H_3-16 or H_3-17), 1.48 (d, J = 6.2 Hz, 3H, H_3-20), 2.51 (d, J = 15.3 Hz, 1H, H-8a), 2.57 (m, 1H, H-15), 2.77 (dd, J = 14.5, 10.1 Hz, 1H, H-18a), 3.22 (m, 2H, H-9, H-18b), 3.58−3.80 (m, 3H, H-8b, H_2-14), 4.31 (m, 1H, H-19), 4.62 (d, J = 10.5 Hz, 1H, H-12), 6.50 (br s, 1H, NH-10), 6.65 (d, J = 7.9 Hz, 1H, H-6 or 7), 6.86 (d, J = 2.1 Hz, 1H, H-2), 6.89 (d, J = 7.9 Hz, 1H, H-6 or 7), 8.03 (br s, 1H, NH-1); minor conformer, δ 0.37 (br s, 3H, H_3-16 or H_3-17), 0.96 (d, J = 6.9 Hz, 3H, H_3-16 or H_3-17), 1.30 (d, J = 5.9 Hz, 3H, H_3-20), 2.15 (br s, 1H, H-15), 2.65 (m, 1H, H-8a), 2.99 (br d, J = 13.5 Hz, 1H, H-8b), 3.06 (dd, 1H, J = 20.9, 10.0 Hz, H-18a), 3.22 (m, 1H, H-18b), 3.58−3.80 (m, 4H, H-12, H_2-14, H-19), 3.91 (br s, 1H, OH-14), 4.22 (br s, 1H, H-9), 6.91 (d, J = 2.0 Hz, 1H, H-2), 6.99 (d, J = 8.1 Hz, 1H, H-6 or 7), 7.03 (d, J = 8.1 Hz, 1H, H-6 or H-7), 7.65 (br d, J = 10.4 Hz, 1H, NH-10), 8.25 (br s, 1H, NH-1), these assignments were derived from a ^{1}H−^{1}H COSY spectrum; HR-EI-MS *m/z* 327.1952 (M^+, calcd for $C_{19}H_{25}N_3O_2$, 327.1948).

Compound **6**: $[\alpha]_D$ −130.8° (c = 0.298, MeOH, 28 °C); UV λ_{max} (EtOH) nm (ϵ) 314 (2000), 301 (2700), 282.5 (5500), 272 (6000), 246.5 (27 000), 243 (26 800); ^{1}H NMR (500 MHz, $CDCl_3$, 0.003 M, 27 °C, twist only) δ 0.20 (d, J = 6.7 Hz, 3H, H_3-16 or 17), 1.04 (d, J = 6.3 Hz, 3H, H_3-16 or H_3-17), 1.72 (br s, 1H, OH-14), 2.58 (d, J = 0.9 Hz, 3H, H_3-20), 2.94 (m, 1H, H-15), 3.05 (dd, J = 17.0, 4.1 Hz, 1H, H-8a), 3.50 (br d, J = 17.0 Hz, 1H, H-8b), 3.55 (m, 1H, H-14a), 3.72 (m, 1H, H-14b), 3.91 (m, 1H, H-9), 5.35 (d, J = 11.0 Hz, 1H, H-12), 6.38 (d, J = 0.9 Hz, 1H, H-18), 6.48 (br s, 1H, NH-10), 6.97 (s, 1H, H-2), 7.11 (d, J = 8.4 Hz, 1H, H-7), 7.29 (d, J = 8.4 Hz, 1H, H-6), 8.09 (br s, 1H, NH-1); ^{13}C NMR (125 MHz, 0.046 M, 27 °C, twist only) δ 14.24 (C-20), 17.09 (C-16 or C-17), 21.27 (C-16 or C-17), 29.16 (C-15), 33.73 (C-8), 55.69 (C-9), 65.07 (C-14), 65.96 (C-12), 105.19 (C-18), 105.74 (C-7), 112.37 (C-3), 112.50 (C-3a), 115.39 (C-6), 120.43 (C-5), 121.16 (C-2), 135.10 (C-7a), 135.17 (C-19), 135.36 (C-4), 173.04 (C-11), these assignments were derived from a ^{1}H−^{1}H COSY, HMQC, and HMBC spectrum; HR-EI-MS *m/z* 325.1780 (M^+, calcd for $C_{19}H_{23}N_3O_2$, 325.1790).

Compound **7**: $[\alpha]_D$ −165.4° (c = 0.104, MeOH, 27 °C); UV λ_{max} (MeOH) nm (ϵ) 297 (5000), 281 (6500), 229 (30 000); ^{1}H NMR (500 MHz, $CDCl_3$, 0.065 M, 27 °C, only one conformer) δ 1.10 (d, J = 6.7 Hz, 3H), 1.26 (d, J = 6.5 Hz, 3H), 2.24 (m, 1H), 2.96 (dd, J = 15.3, 9.4 Hz, 1H), 3.18 (ddd, J = 15.3, 7.0, 0.8 Hz, 1H), 3.37 (d, J = 9.7 Hz, 1H), 3.41 (m, 1H), 3.48 (dd, J = 16.7, 6.3 Hz, 1H), 3.54 (br s, 1H, NH-13), 3.63 (dd, J = 11.2, 5.9 Hz, 1H), 3.78 (dd, J = 11.2, 3.8 Hz, 1H), 4.96 (dd, J = 17.2, 1.7 Hz, 1H), 5.07 (dd, J = 10.2, 1.7 Hz, 1H), 5.70 (br d, J = 11.3 Hz, 1H, NH-10), 6.00 (m, 2H), 6.77 (s, 1H), 6.92 (d, J = 8.2 Hz, 1H), 6.96 (d, J = 8.2 Hz, 1H), 7.95 (br s, 1H, NH-1); HR-EI-MS *m/z* 345.1596 (M^+, calcd for $C_{19}H_{24}N_3OCl$, 345.1608).

Compound **8**: $[\alpha]_D$ −39.1° (c = 0.10, MeOH, 25 °C); UV λ_{max} (EtOH) nm (ϵ) 300.5 (5300), 291 (6800), 283 (7500), 229 (31 000); ^{1}H NMR (500 MHz, $CDCl_3$, 0.032 M, 27 °C, only one conformer) δ 1.16 (d, J = 7.1 Hz, 3H, H_3-16 or H_3-17), 1.19 (s, 3H, H_3-14), 1.29 (d, J = 6.7 Hz, 3H, H_3-16 or H_3-17), 2.19 (dsep, J = 6.9, 2.4 Hz, 1H, H-15), 2.89 (d, J = 15.3 Hz, 1H, H-8a), 3.16 (br d, J = 15.3 Hz, 1H, H-8b), 3.32 (d, J = 2.5 Hz, 1H, H-11), 3.41 (dd, J = 15.4, 7.1 Hz, 1H, H-18a), 3.73 (dd, J = 15.4, 5.7 Hz, 1H, H-18b), 5.03 (dd, J = 10.0, 1.4 Hz, H-20a), 5.08 (dd, J = 17.1, 1.7 Hz, H-20b), 5.92 (m, 1H, H-19), 6.25 (br s, 1H, NH-13), 6.87 (t, J = 1.7 Hz, 1H, H-2), 7.08 (d, J = 8.3 Hz, 1H, H-6), 7.15 (d, J = 8.3 Hz, 1H, H-7), 7.96 (br s, 1H, NH-1); ^{13}C NMR (125 MHz, 0.032 M, 27 °C, only one conformer) δ 15.74 (C-16 or C-17), 19.60 (C-16 or C-17), 24.85 (C-14), 26.40 (C-15), 32.02 (C-8), 34.59 (C-18), 70.87 (C-11), 73.96 (C-9), 108.19 (C-7), 108.86 (C-3), 115.04 (C-20), 118.08 (C-2), 121.42 (C-3a), 126.10 (C-6), 126.79 (C-5), 133.48 (C-7a), 134.67 (C-4), 138.88 (C-19), 174.66 (C-12), these assignments were derived from a ^{1}H−^{1}H COSY, NOESY, C−H COSY, and COLOC spectrum; HR-EI-MS *m/z* 309.1861 (M^+, calcd for $C_{19}H_{23}N_3O$, 309.1841).

Synthesis of 6 by DDQ Oxidation. Compound **5b** (4.0 mg, 12 μmol) was acetylated in pyridine (0.25 mL) and acetic anhydride (0.25 mL). After 50 min, ice was added to the reaction mixture, which was evaporated *in vacuo* to dryness. The residue was purified by column chromatography on Wakogel C-100 using toluene and increasing amounts of acetone to give the 14-*O*-acetate of **5b** quantitatively. DDQ (4.2 mg, 19 μmol) in dioxane (0.4 mL) was added to the dioxane solution of this acetate (0.8 mL). After being stirred for 2.5 h, the reaction mixture was evaporated to dryness and applied to a column of neutral alumina. Elution with EtOAc, followed by alkaline treatment (1 N KOH in MeOH), gave crude **6**, which was purified by HPLC on YMC AQ-323 using 60% MeOH to give **6** (2.8 mg, 8.6 μmol) in 70% yield.

Synthesis of 14-*O*-Acetate of 6 (9) by Palladium-Assisted Intramolecular Amination. Compound **4** (117.6 mg, 0.360 mmol) was treated with acetic anhydride (0.5 mL) in pyridine (0.5 mL). After being stirred at room temperature for 45 min, the reaction mixture was evaporated with toluene *in vacuo* to dryness. The residue was purified by column chromatography on Wakogel C-100 using toluene and increasing amounts of acetone to give the 14-*O*-acetate of **4** quantitatively. $PdCl_2(CH_3CN)_2$ (7.9 mg, 30 μmol), benzoquinone (33.8 mg, 0.313 mmol), and LiCl (130.3 mg, 3.07 mmol) was dissolved in anhydrous THF (4.2 mL). The THF solution of the 14-*O*-acetate of **4** (112.3 mg, 0.304 mmol) was added to the solution. After being stirred for 2.5 h at room temperature, the reaction mixture was concentrated *in vacuo* to dryness. The residue was purified by column chromatography on Wakogel C-100 using toluene and increasing amounts of acetone, followed by HPLC on μ-Bondasphere using 70% MeOH and on YMC A-023 using 85% *n*-hexane, 10% $CHCl_3$, and 5% 2-PrOH to give **9** (74.4 mg, 0.203 mmol) in 67% yield: $[\alpha]_D$ −126.3° (c = 0.69, MeOH, 26 °C); UV λ_{max} (MeOH) nm (ϵ) 313 (2400), 302 (3400), 282 (7100), 271 (8100), 246 (35 800), 243 (36 000); ^{1}H NMR (500 MHz, $CDCl_3$, 0.054 M, 27 °C, twist only) δ 0.21 (d, J = 6.7 Hz, 3H, H_3-16 or H_3-17), 1.04 (d, J = 6.3 Hz, 3H, H_3-16 or H_3-17), 2.05 (s, 3H, Ac), 2.59 (d, J = 0.8 Hz, 3H, H_3-20), 2.95 (m, 1H, H-15), 3.15 (dd, J = 17.1, 4.3 Hz, 1H, H-8a), 3.55 (br d, J = 17.1 Hz, 1H, H-8b), 4.00 (dd, J = 11.0, 8.2 Hz, 1H, H-14a), 4.10 (m, 1H, H-9), 4.14 (dd, J = 11.0, 3.5 Hz, 1H, H-14b), 5.34 (d, J = 10.9 Hz, 1H, H-12), 6.13 (br s, 1H, NH-10), 6.39 (d, J = 0.8 Hz, 1H, H-18), 6.98 (dd, J = 2.2, 1.1 Hz, 1H, H-2), 7.11 (d, J = 8.5 Hz, 1H, H-7), 7.29 (d, J = 8.5 Hz, 1H, H-6), 8.21 (br s, 1H, NH-1); ^{13}C NMR (125 MHz, 0.054 M, 27 °C, twist only) δ 14.28 (C-20), 17.11 (C-16 or C-17), 20.76 (Ac), 21.29 (C-16 or C-17), 29.27 (C-15), 34.26 (C-8), 53.11 (C-9), 65.93 (C-14), 66.05 (C-12), 105.28 (C-18), 105.84 (C-7), 111.58 (C-3), 112.38 (C-3a), 115.53 (C-6), 120.54 (C-5), 121.23 (C-2), 135.16 (C-7a), 135.28 (C-4), 135.35 (C-19), 170.94 (Ac), 172.15 (C-11), these assignments were derived from a ^{1}H−^{1}H COSY, C−H COSY, and COLOC spectrum; EI-MS *m/z* 367 (M^+).

Synthesis of 18 and 19 by Palladium-Assisted Intramolecular Amination. Since the corresponding N-substituted substrates of **16** and **17** in the aza-Claisen rearrangement could not be synthesized, direct substitution of (−)-*N*^{13}-desmethylindolactam-V (**2**) by crotyl bromide

or 1-bromo-2-pentene was carried out in 13 N acetic acid and sodium acetate by a method similar to that reported previously.[31] Compound **16** was obtained in 7.9% yield by HPLC on μ-Bondasphere using 42.5% CH_3CN, followed on YMC A-023 using 75% *n*-hexane, 10% $CHCl_3$, and 15% 2-PrOH and on μ-Bondasphere using 35% CH_3CN: $[\alpha]_D$ −203.8° (c = 0.365, MeOH, 19 °C); UV λ_{max} (MeOH) nm (ϵ) 298 (4600), 282.5 (6000), 229.5 (30 400); ^{1}H NMR (500 MHz, $CDCl_3$, 0.086 M, 27 °C, only one conformer) δ 1.04 (d, J = 6.7 Hz, 3H), 1.23 (d, J = 6.4 Hz, 3H), 1.65 (d, J = 5.9 Hz, 3H), 2.21 (m, 1H), 2.80 (dd, J = 15.1, 9.3 Hz, 1H), 2.97 (dd, J = 15.1, 6.8 Hz, 1H), 3.25−3.65 (m, 7H), 5.30 (m, 1H), 5.40 (m, 1H), 5.61 (m, 1H), 6.19 (br s, 1H, NH-10), 6.57 (s, 1H), 6.91 (s, 2H), 8.07 (br s, 1H, NH-1); HR-EI-MS *m/z* 341.2119 (M^+, calcd for $C_{20}H_{27}N_3O_2$, 341.2103).

Compound **17** was obtained in 10.1% yield by column chromatography on Wakogel C-100 using toluene and increasing amounts of acetone, followed by HPLC on μ-Bondasphere using 42.5% CH_3CN: $[\alpha]_D$ −168.6° (c = 0.587, MeOH, 27 °C); UV λ_{max} (MeOH) nm (ϵ) 298 (4100), 281.5 (5400), 230 (26 100); ^{1}H NMR (500 MHz, $CDCl_3$, 0.056 M, 27 °C, only one conformer) δ 0.96 (t, J = 7.4 Hz, 3H), 1.05 (d, J = 6.7 Hz, 3H), 1.24 (d, J = 6.5 Hz, 3H), 2.01 (m, 2H), 2.22 (m, 1H), 2.83 (dd, J = 15.3, 9.3 Hz, 1H), 3.01 (dd, J = 15.3, 7.1 Hz, 1H), 3.02 (br s, 1H, OH-14), 3.30−3.75 (m, 6H), 5.35 (br s, 1H), 5.46 (m, 1H), 5.58 (m, 1H), 6.14 (br d, J = 9.8 Hz, 1H, NH-10), 6.64 (s, 1H), 6.92 (d, J = 8.2 Hz, 1H), 6.95 (d, J = 8.2 Hz, 1H), 8.00 (br s, 1H, NH-1); HR-EI-MS *m/z* 355.2289 (M^+, calcd for $C_{21}H_{29}N_3O_2$, 355.2260).

Compound **16** (14.6 mg, 42.8 μmol) was treated in a manner similar to that described for the synthesis of **9** to give **18** (3.5 mg, 10 μmol) in 24% yield: $[\alpha]_D$ −171.7° (c = 0.158, MeOH, 24 °C); UV λ_{max} (MeOH) nm (ϵ) 313 (2500), 301.5 (3400), 282.5 (6600), 271.5 (7800), 247.5 (33 100), 243.5 (33 000); ^{1}H NMR (500 MHz, $CDCl_3$, 0.021 M, 27 °C, twist only) δ 0.14 (d, J = 6.7 Hz, 3H), 1.02 (d, J = 6.3 Hz, 3H), 1.31 (t, J = 7.4 Hz, 3H), 2.14 (br t, J = 5.6 Hz, 1H, OH-14), 2.83−3.04 (m, 3H), 3.07 (dd, J = 17.1, 4.1 Hz, 1H), 3.46 (br d, J = 17.1 Hz, 1H), 3.54 (m, 1H), 3.65 (m, 1H), 3.91 (m, 1H), 5.34 (d, J = 10.9 Hz, 1H), 6.45 (s, 1H), 6.83 (br s, 1H, NH-10), 6.94 (d, J = 0.8 Hz, 1H), 7.09 (d, J = 8.4 Hz, 1H), 7.30 (d, J = 8.4 Hz, 1H), 8.07 (br s, 1H, NH-1); HR-EI-MS *m/z* 339.1963 (M^+, calcd for $C_{20}H_{25}N_3O_2$, 339.1947).

Compound **17** (10.1 mg, 28.5 μmol) was treated in a manner similar to that described for the synthesis of **9** to give **19** (0.3 mg, 0.8 μmol) in 3.0% yield: $[\alpha]_D$ −290.4° (c = 0.0135, MeOH, 22 °C); UV λ_{max} (MeOH) nm (ϵ) 314 (3100), 302 (4200), 283 (8100), 271.5 (9500), 247.5 (42 500), 243 (42 100); ^{1}H NMR (500 MHz, $CDCl_3$, 0.0021 M, 27 °C, twist only) δ 0.13 (d, J = 6.7 Hz, 3H), 1.02 (m, 6H), 1.73 (m, 2H), 2.87 (m, 2H), 3.01 (m, 1H), 3.06 (dd, J = 17.1, 4.2 Hz, 1H), 3.48 (br d, J = 17.1 Hz, 1H), 3.55 (m, 1H), 3.70 (m, 1H), 4.00 (m, 1H), 5.31 (d, J = 10.9 Hz, 1H), 6.38 (br s, 1H, NH-10), 6.45 (s, 1H), 6.97 (s, 1H), 7.12 (d, J = 8.4 Hz, 1H), 7.31 (d, J = 8.4 Hz, 1H), 8.09 (br s, 1H, NH-1); HR-EI-MS *m/z* 353.2110 (M^+, calcd for $C_{21}H_{27}N_3O_2$, 353.2103).

Synthesis of (−)-5-Methylindolactam-V (22). $AlCl_3$ (28 mg, 210 μmol) and $LiAlH_4$ (6 mg, 160 μmol) dissolved in anhydrous THF (1 mL) were added to an anhydrous THF solution (1 mL) of (−)-14-*O*-acetyl-5-formylindolactam-V[30] (7.2 mg, 19 μmol). After being refluxed for 10 min, the reaction mixture was quenched with EtOAc, and extracted with EtOAc. The EtOAc layer was dried over Na_2SO_4 and evaporated *in vacuo* to dryness. The residue was purified by column chromatography on Wakogel C-100 using toluene and increasing amounts of acetone, followed by HPLC on YMC AQ-323 using 70% MeOH to give **22** (3.4 mg, 11 μmol) in 56% yield: $[\alpha]_D$ +86.2° (c = 0.11, MeOH, 17 °C); UV λ_{max} (EtOH) nm (ϵ) 290 (6300), 226 (24 900); ^{1}H NMR (500 MHz, $CDCl_3$, 0.022 M, 27 °C, sofa >98%) δ 0.97 (d, J = 6.5Hz, 3H), 1.29 (d, J = 7.0 Hz, 3H), 2.46 (s, 3H), 2.65 (m, 1H), 2.73 (s, 3H), 2.78 (dd, J = 14.3, 1.5 Hz, 1H), 2.99 (d, J = 10.7 Hz, 1H), 3.21 (dd, J = 14.3, 4.3 Hz, 1H), 3.37 (m, 2H), 4.47 (m, 1H), 4.91 (d, J = 10.7 Hz, 1H, NH-10), 7.01 (d, J = 2.5 Hz, 1H), 7.08 (d, J = 8.2 Hz, 1H), 7.18 (d, J = 8.2 Hz, 1H), 8.22 (br s, 1H, NH-1); HR-EI-MS *m/z* 315.1933 (M^+, calcd for $C_{18}H_{25}N_3O_2$, 315.1948).

Prenylation of 9 and 5a. Compound **9** (68.9 mg, 0.188 mmol) was dissolved in 13 N acetic acid (0.36 mL) and sodium acetate (35.9 mg, 0.417 mmol). Prenyl bromide (26.9 μL, 0.233 mmol) was added to the mixture, which was stirred at room temperature for 30 min. After neutralization with aqueous $NaHCO_3$, 1 N NaOH aqueous solution (2 mL) and MeOH (2 mL) were added to the mixture. After being stirred for 40 min, the reaction mixture was extracted with EtOAc. The EtOAc extracts were purified by column chromatography on Wakogel C-100 using toluene and increasing amounts of acetone, followed by HPLC on μ-Bondasphere using 70% MeOH and on YMC A-023 using 82.5% *n*-hexane, 10% $CHCl_3$, and 7.5% 2-PrOH to give **10** (6.1 mg, 16 μmol), **11** (1.3 mg, 3.3 μmol), and **12** (5.1 mg, 13 μmol) in 8.3, 1.8, and 6.9% yields, respectively.

Compound **10**: $[\alpha]_D$ −109.0° (c = 0.104, MeOH, 29 °C); UV λ_{max} (MeOH) nm (ϵ) 312 (2600), 301 (4800), 285.5 (10 000), 274.5 (9500), 246 (39 700); ^{1}H NMR (500 MHz, $CDCl_3$, 0.01 M, 27 °C, twist only) δ 0.17 (d, J = 6.7 Hz, 3H), 1.03 (d, J = 6.3 Hz, 3H), 1.78 (s, 3H), 1.80 (s, 3H), 1.98 (br s, 1H, OH-14), 2.54 (s, 3H), 2.91 (m, 1H), 3.07 (dd, J = 17.1, 4.2 Hz, 1H), 3.32 (br d, J = 17.1 Hz, 1H), 3.46 (m, 2H), 3.53 (m, 1H), 3.63 (m, 1H), 3.96 (br s, 1H), 5.31 (br t, J = 7.3 Hz, 1H), 5.35 (d, J = 10.9 Hz, 1H), 6.35 (s, 1H), 6.72 (br s, 1H, NH-10), 7.05 (d, J = 8.4 Hz, 1H), 7.21 (d, J = 8.4 Hz, 1H), 7.93 (br s, 1H, NH-1); HR-EI-MS *m/z* 393.2413 (M^+, calcd for $C_{24}H_{31}N_3O_2$, 393.2416).

Compound **11**: $[\alpha]_D$ −245.9° (c = 0.067, MeOH, 25 °C); UV λ_{max} (MeOH) nm (ϵ) 315 (3700), 303 (4400), 283 (6800), 272 (8700), 247 (38 900), 244.5 (38 900); ^{1}H NMR (500 MHz, $CDCl_3$, 0.0076 M, 27 °C, twist only) δ 0.21 (d, J = 6.7 Hz, 3H), 1.03 (d, J = 6.3 Hz, 3H), 1.79 (s, 3H), 1.83 (s, 3H), 1.95 (m, 1H, OH-14), 2.56 (s, 3H), 2.93 (m, 1H), 3.06 (dd, J = 17.0, 4.1 Hz, 1H), 3.49 (br d, J = 17.0 Hz, 1H), 3.55 (m, 3H), 3.67 (m, 1H), 3.91 (br s, 1H), 5.32 (d, J = 10.9 Hz, 1H), 5.43 (br t, J = 7.0 Hz, 1H), 6.33 (s, 1H), 6.63 (br s, 1H, NH-10), 6.96 (s, 1H), 7.09 (s, 1H), 8.08 (br s, 1H, NH-1); HR-EI-MS *m/z* 393.2422 (M^+, calcd for $C_{24}H_{31}N_3O_2$, 393.2416).

Compound **12**: $[\alpha]_D$ −157.5° (c = 0.141, MeOH, 27 °C); UV λ_{max} (MeOH) nm (ϵ) 315 (2300), 303 (3900), 286.5 (8500), 275 (8600), 249 (37 000); ^{1}H NMR (500 MHz, $CDCl_3$, 0.01 M, 27 °C, twist only) δ 0.13 (d, J = 6.7 Hz, 3H), 1.02 (d, J = 6.3 Hz, 3H), 1.68 (s, 3H), 1.82 (s, 3H), 2.04 (m, 1H, OH-14), 2.50 (s, 3H), 2.87 (m, 1H), 3.05 (dd, J = 17.0, 4.0 Hz, 1H), 3.41 (m, 2H), 3.46 (br d, J = 17.0 Hz, 1H), 3.53 (m, 1H), 3.65 (m, 1H), 3.93 (br s, 1H), 5.24 (br t, J = 6.9 Hz, 1H), 5.28 (d, J = 10.9 Hz, 1H), 6.75 (br s, 1H, NH-10), 6.93 (s, 1H), 7.09 (d, J = 8.4 Hz, 1H), 7.27 (d, J = 8.4 Hz, 1H), 8.05 (br s, 1H, NH-1); HR-EI-MS *m/z* 393.2399 (M^+, calcd for $C_{24}H_{31}N_3O_2$, 393.2416).

Compound **5a** (8.9 mg, 27 μmol) was dissolved in 13 N acetic acid (0.1 mL) and sodium acetate (5.2 mg, 63 μmol). Prenyl bromide (3.5 μL, 30 μmol) was added to the mixture, which was stirred at room temperature for 15 min. After neutralization with aqueous $NaHCO_3$, the reaction mixture was extracted with EtOAc. The EtOAc extracts were purified by column chromatography on Wakogel C-100 using toluene and increasing amounts of acetone, followed by HPLC on YMC AQ-323 using 75% MeOH and on YMC A-023 using 85% *n*-hexane, 10% $CHCl_3$, and 5% 2-PrOH to give **13** (0.4 mg, 1 μmol), **14** (2.0 mg, 5.1 μmol), and **15** (1.3 mg, 3.2 μmol) in 3.7, 19, and 12% yields, respectively.

Compound **13**: $[\alpha]_D$ −212.8° (c = 0.0218, MeOH, 20 °C); UV λ_{max} (MeOH) nm (ϵ) 320 (8200), 290 (6200), 239 (24 200); ^{1}H NMR (500 MHz, $CDCl_3$, 0.002 M, 27 °C, twist only) δ 0.72 (d, J = 6.6 Hz, 3H), 1.00 (d, J = 6.3 Hz, 3H), 1.18 (d, J = 6.3 Hz, 3H), 1.71 (br s, 1H, OH-14), 1.75 (s, 3H), 1.78 (s, 3H), 2.55 (dd, J = 15.3, 3.3 Hz, 1H), 2.64 (m, 1H), 2.90 (dd, J = 17.2, 4.5 Hz, 1H), 3.04 (br d, J = 17.2 Hz, 1H), 3.33 (dd, J = 16.3, 7.0 Hz, 1H), 3.40 (dd, J = 16.3, 7.3 Hz, 1H), 3.51 (m, 1H), 3.64 (m, 2H), 4.02 (m, 1H), 4.27 (br s, 1H), 4.32 (d, J = 10.5 Hz, 1H), 5.26 (br t, J = 7.2 Hz, 1H), 6.24 (br s, 1H, NH-10), 6.65 (d, J = 8.0 Hz, 1H), 6.84 (d, J = 8.0 Hz, 1H), 7.65 (br s, 1H, NH-1); HR-EI-MS *m/z* 395.2565 (M^+, calcd for $C_{24}H_{33}N_3O_2$, 395.2573).

Compound **14**: $[\alpha]_D$ −329.2° (c = 0.0888, MeOH, 19 °C); UV λ_{max} (MeOH) nm (ϵ) 324 (9400), 317.5 (8800), 288.5 (7700), 235 (25 200); ^{1}H NMR (500 MHz, $CDCl_3$, 0.0099 M, 27 °C, twist only) δ 0.72 (d, J = 6.2 Hz, 3H), 0.99 (d, J = 6.2 Hz, 3H), 1.16 (d, J = 6.3 Hz, 3H), 1.77 (s, 3H), 1.80 (s, 3H), 2.20 (br s, 1H, OH-14), 2.55 (br d, J = 14.9 Hz, 1H), 2.64 (m, 1H), 2.92 (dd, J = 17.0, 3.8 Hz, 1H), 3.20 (br d, J = 17.0 Hz, 1H), 3.43 (m, 2H), 3.56 (m, 1H), 3.62 (m, 1H), 3.70 (m, 1H), 4.01 (m, 1H), 4.28 (m, 2H), 5.37 (br t, J = 7.0 Hz, 1H), 6.60 (br s, 1H, NH-10), 6.72 (s, 1H), 6.85 (s, 1H), 7.80 (br s, 1H, NH-1); HR-EI-MS *m/z* 395.2587 (M^+, calcd for $C_{24}H_{33}N_3O_2$, 395.2573).

Compound **15**: $[\alpha]_D$ −324.8° (c = 0.0458, MeOH, 22 °C); UV λ_{max} (MeOH) nm (ϵ) 322 (10 500), 316.5 (10 200), 288 (8000), 235 (24 300); ^{1}H NMR (500 MHz, $CDCl_3$, 0.0079 M, 27 °C, twist only) δ 0.72 (d, J = 6.5 Hz, 3H), 0.98 (d, J = 6.2 Hz, 3H), 1.18 (d, J = 6.3 Hz, 3H), 1.46 (s, 3H), 1.47 (s, 3H), 2.08 (br s, 1H, OH-14), 2.57 (d, J = 14.1 Hz, 1H), 2.64 (m, 1H), 2.90 (dd, J = 17.0, 4.0 Hz, 1H), 3.19 (br d, J = 17.0 Hz, 1H), 3.54 (m, 1H), 3.64 (dd, J = 14.1, 10.6 Hz, 1H), 3.70 (m, 1H), 4.03 (m, 1H), 4.28 (m, 2H), 5.19 (d, J = 10.6 Hz, 1H), 5.30 (d, J = 17.6 Hz, 1H), 6.18 (dd, J = 17.6, 10.6 Hz, 1H), 6.51 (br s, 1H, NH-10), 6.79 (s, 1H), 6.86 (s, 1H), 8.29 (br s, 1H, NH-1); HR-EI-MS *m/z* 395.2584 (M^+, calcd for $C_{24}H_{33}N_3O_2$, 395.2573).

Aza-Claisen Rearrangement of (+)-Epi-*N*^{13}-desmethyl-*N*^{13}-allylindolactam-V (25). **25** was obtained from (+)-*N*^{13}-desmethylindolactam-V[6] in a manner similar to that described for **3** in 53% yield: $[\alpha]_D$ +167.4° (c = 0.867, MeOH, 26 °C); UV λ_{max} (MeOH) nm (ϵ) 302 (7300), 289 (6500), 229.5 (22 800); ^{1}H NMR (500 MHz, $CDCl_3$, 0.11 M, 27 °C, *r*-*cis*-sofa:*r*-sofa = 7.3:1) *r*-*cis*-sofa conformer, δ 0.74 (d, J = 6.4 Hz, 3H, H_3-16 or H_3-17), 0.78 (d, J = 6.6 Hz, 3H, H_3-16 or H_3-17), 2.60 (m, 1H, H-15), 2.94 (br d, J = 15.5 Hz, 1H, H-8a), 3.32 (br d, J = 15.5 Hz, 1H, H-8b), 3.77 (br s, 1H, OH-14), 3.85 (br s, 3H, H-9, H_2-14), 3.95 (d, J = 10.5 Hz, 1H, H-12), 4.18 (dd, J = 15.8, 7.0 Hz, 1H, H-18a), 4.52 (br d, J = 15.8 Hz, 1H, H-18b), 4.96 (d, J = 10.3 Hz, 1H, H-20a), 5.17 (d, J = 17.2 Hz, 1H, H-20b), 5.74 (m, 1H, H-19), 6.77 (d, J = 7.5 Hz, 1H, H-5), 6.87 (d, J = 1.9 Hz, 1H, H-2), 6.95 (d, J = 7.9 Hz, 1H, H-7), 7.00 (t, J = 7.7 Hz, 1H, H-6), 7.77 (br s, 1H, NH-10), 8.02 (br s, 1H, NH-1); r-sofa conformer, δ 0.94 (d, J = 6.5 Hz, 3H, H_3-16 or H_3-17), 1.29 (d, J = 6.6 Hz, 3H, H_3-16 or H_3-17), 2.36 (m, 1H, H-15), 2.81 (br d, J = 14.2 Hz, 1H, H-8a), 3.05 (d, J = 10.9 Hz, 1H, H-12), 3.18 (dd, J = 14.2, 11.7 Hz, 1H, H-8b), 3.51 (dd, J = 15.1, 6.8 Hz, 1H, H-14a), 4.74 (d, J = 10.0 Hz, 1H, H-20a), 4.81 (d, J = 17.1 Hz, 1H, H-20b), 5.67 (m, 1H, H-19), 7.04 (d, J = 7.8 Hz, 1H, H-5), 7.08 (d, J = 2.0 Hz, 1H, H-2), 7.15 (t, J = 7.7 Hz, 1H, H-6), 7.28 (d, J = 8.3 Hz, 1H, H-7), 8.37 (br s, 1H, NH-1), other peaks of the *r*-sofa conformer overlapped the peaks of the *r*-*cis*-sofa conformer and/or solvent, and these assignments were derived from a ^{1}H−^{1}H COSY spectrum; HR-EI-MS *m/z* 327.1959 (M^+, calcd for $C_{19}H_{25}N_3O_2$, 327.1947).

Compound **25** was treated in a manner similar to that described for the aza-Claisen rearrangement of **3** to give *ortho* rearrangement products, **26a**, **26b**, and **27** in 4.7, 4.1, and 21.7% yields, respectively.

Compound **26a**: $[\alpha]_D$ +114.4° (c = 0.146, MeOH, 24 °C); UV λ_{max} (MeOH) nm (ϵ) 319 (2800), 307.5 (5500), 298 (5300), 283 (5500), 232.5 (25 200); ^{1}H NMR (500 MHz, CD_3OD, 0.021 M, 27 °C, major: minor = 2.6:1) major conformer, δ 0.93 (d, J = 6.8 Hz, 3H), 1.17 (d, J = 6.5 Hz, 3H), 1.19 (d, J = 6.9 Hz, 3H), 2.37 (d, J = 15.0 Hz, 1H), 2.45 (m, 1H), 2.76 (dd, J = 14.3, 12.0 Hz, 1H), 2.90 (d, J = 5.4 Hz, 1H), 3.02 (dd, J = 14.3, 1.9 Hz, 1H), 3.44 (dd, J = 15.0, 6.5 Hz, 1H), 3.69 (dd, J = 11.0, 5.6 Hz, 1H), 3.76 (dd, J = 11.0, 4.2 Hz, 1H), 3.99 (m, 2H), 7.03 (d, J = 8.1 Hz, 1H), 7.04 (s, 1H), 7.12 (d, J = 8.1 Hz, 1H); minor conformer, δ 0.68 (d, J = 6.5 Hz, 3H), 0.86 (d, J = 6.5 Hz, 3H), 1.22 (d, J = 6.4 Hz, 3H), 2.50 (m, 2H), 2.95 (dd, J = 15.9, 4.6 Hz, 1H), 3.05 (dd, J = 15.9, 2.2 Hz, 1H), 3.60 (dd, J = 15.1, 9.3 Hz, 1H), 3.69 (m, 2H), 3.92 (br s, 1H), 4.11 (m, 1H), 4.22 (d, J = 10.6 Hz, 1H), 6.75 (d, J = 8.1 Hz, 1H), 6.80 (d, J = 8.1 Hz, 1H), 6.92 (s, 1H); HR-EI-MS *m/z* 327.1967 (M^+, calcd for $C_{19}H_{25}N_3O_2$, 327.1947).

Compound **26b**: $[\alpha]_D$ −137.8° (c = 0.152, MeOH, 22 °C); UV λ_{max} (MeOH) nm (ϵ) 320.5 (6600), 315 (6900), 288 (5300), 234 (22 600); ^{1}H NMR (500 MHz, $CDCl_3$, 0.019 M, 27 °C, only one conformer) δ 1.01 (d, J = 5.4 Hz, 3H), 1.04 (d, J = 6.6 Hz, 3H), 1.35 (d, J = 6.0 Hz, 3H), 2.50 (m, 1H), 2.58 (dd, J = 14.7, 6.5 Hz, 1H), 2.76 (d, J = 16.3 Hz, 1H), 3.07 (br s, 1H, OH-14), 3.43 (m, 3H), 3.58 (m, 1H), 4.14 (m, 2H), 4.34 (d, J = 9.1 Hz, 1H), 6.73 (d, J = 7.9 Hz, 1H), 6.74 (s, 1H), 6.91 (d, J = 7.9 Hz, 1H), 6.98 (br s, 1H, NH-10), 7.80 (br s, 1H, NH-1); HR-EI-MS *m/z* 327.1973 (M^+, calcd for $C_{19}H_{25}N_3O_2$, 327.1947).

Compound **27**: $[\alpha]_D$ +38.1° (c = 0.537, MeOH, 24 °C); UV λ_{max} (MeOH) nm (ϵ) 301 (5300), 284.5 (6000), 230 (27 900); the ^{1}H NMR signals of **27** were very broad at 27 °C and could not be analyzed; HR-EI-MS *m/z* 327.1959 (M^+, calcd for $C_{19}H_{25}N_3O_2$, 327.1947).

Synthesis of 28 by Palladium-Assisted Intramolecular Amination. Compound **27** (16.3 mg, 49.8 μmol) was treated in a manner similar to that described for the synthesis of **9** to give **28** (6.3 mg, 19 μmol) in 39% yield: $[\alpha]_D$ +120.0° (c = 0.289, MeOH, 25 °C); UV λ_{max} (MeOH) nm (ϵ) 313 (1800), 300.5 (2900), 282 (6900), 271.5 (8300), 241.5 (28 500); ^{1}H NMR (500 MHz, $CDCl_3$, 0.025 M, 27 °C, *r*-twist only) δ 0.96 (d, J = 6.0 Hz, 3H), 1.16 (d, J = 6.1 Hz, 3H), 2.55 (br s, 1H, OH-14), 2.70 (s, 3H), 3.07 (m, 3H), 3.35 (m, 1H), 3.77 (br s, 1H), 3.89 (br d, J = 15.0 Hz, 1H), 5.67 (d, J = 11.0 Hz, 1H), 6.31 (s, 1H), 6.40 (br s, 1H, NH-10), 6.76 (s, 1H), 6.98 (d, J = 8.2 Hz, 1H), 7.26 (d, J = 8.2 Hz, 1H), 8.02 (br s, 1H, NH-1); HR-EI-MS *m/z* 325.1785 (M^+, calcd for $C_{19}H_{23}N_3O_2$, 325.1790).

Inhibition of Specific [^{3}H]PDBu Binding to γ-Cys2 or η-Cys2. The standard mixture (250 μL) contained in a 1.5 mL Eppendorf tube 50 mM Tris-HCl (pH 7.4 at 25 °C), 10 nM γ-Cys2, 30 nM [^{3}H]PDBu (20 Ci/mmol), 50 μg/mL 1,2-di(*cis*-9-octadecenoyl)-*sn*-glycero-3-phospho-L-serine, 3 mg/mL bovine γ-globulin, and various concentrations of an inhibitor. Final DMSO concentration was 2%. For the η-Cys2 binding assay, 20 nM [^{3}H]PDBu and 5 nM η-Cys2 were used. The phosphatidylserine was suspended in 50 mM Tris-HCl (pH 7.4) by sonication (1 min) and added to the above reaction mixture. Total binding was measured in the absence of an inhibitor, and nonspecific binding was measured in the presence of 500-fold of nonradioactive PDBu. Specific binding represents the difference between the total and the nonspecific binding. The samples were then incubated at 30 °C for 20 min. After the mixture was cooled at 0 °C for 5 min, 187 μL of 35% (w/w) poly(ethylene glycol) (average molecular weight, 8000) was added to the tubes and the mixture was vigorously stirred. The tubes were incubated at 0 °C for 15 min and centrifuged for 20 min at 12 000 rpm in an Eppendorf microcentrifuge at 4 °C. The supernatant of each tube was removed by aspiration, and the tube was blotted with a Kimwipe. The tip of the tube was cut off, and the radioactivity in the pellet was measured to determine the bound [^{3}H]-PDBu. In each experiment, each point represents the average of at least triplicate determinations. Binding affinity was evaluated by the concentration required to cause 50% inhibition of the specific binding, IC_{50}, which was calculated by a computer program (SAS) with a probit procedure.[41] The binding constant, K_i, was calculated by the method of Sharkey and Blumberg.[42]

Epstein−Barr Virus Early Antigen Induction Test. The EBV-EA induction test was done in the Raji cell (nonproducer) system with sodium *n*-butyrate (3 mM) by the method reported previously.[46,47]

Stimulation of $^{32}P_i$ Incorporation into HeLa Cell Phospholipids. Incorporation of $^{32}P_i$ into HeLa cell phospholipids was measured by the method reported previously[49] with slight modifications.[51]

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Supporting Information Available: ^{1}H NMR and NOESY spectra of **5a**, **6**, **11**, and **14** and the 1D NOE spectra of **5a**, **6**, and **28** (16 pages). See any current masthead page for ordering and Internet access instructions.

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